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Cell Biology

Cell–cell adhesion mediated by binding of membrane-anchored transforming growth factor α to epidermal growth factor receptors promotes cell proliferation

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ABSTRACT **The precursor for transforming growth factor α, pro-TGF-α, is a cell surface glycoprotein that can establish contact with epidermal growth factor (EGF) receptors on adjacent cells. To examine whether the pro-TGF-α/EGF receptor pair can simultaneously mediate cell adhesion and promote cell proliferation, we have expressed pro-TGF-α in a bone marrow stromal cell line. Expression of pro-TGF-α allows these cells to support long-term attachment of an EGF/interleukin-3-dependent hematopoietic progenitor cell line that expresses EGF receptors but is unable to adhere to normal stroma. This interaction is inhibited by soluble EGF receptor ligands. Further, the hematopoietic progenitor cells replicate their DNA while they are attached to the stromal cell layer and become foci of sustained cell proliferation. Thus, pro-TGF-α and the EGF receptor can function as mediators of intercellular adhesion and this interaction may promote a mitogenic response. We propose the term "juxtacrine" to designate this form of stimulation between adjacent cells.**

Several growth and morphogenesis factors are produced as part of membrane-anchored precursors that may interact with receptors located on the surface of adjacent cells. Factors that derive from membrane precursors include the members of the epidermal growth factor (EGF) family. In addition to EGF (1, 2), this family includes transforming growth factor α (TGF-α) (3, 4); vaccinia growth factor, encoded by vaccinia virus (5); the products of two *Drosophila* neurogenic genes, Notch and Delta (6, 7); and the products of two genes, *lin-12* and *glp-1*, that determine cell fate in the nematode *Caenorhabditis elegans* (8–10). These molecules are synthesized as polypeptides that contain one, in the case of TGF-α and vaccinia growth factor precursors, or multiple copies of the consensus "EGF-like" structure. This structure, defined by six characteristically spaced cysteines, is ≈50 amino acids long and can be released as a diffusible bioactive factor. The membrane precursors consist of an extracellular domain that contains the EGF-like repeats followed by a membrane-spanning hydrophobic sequence and a cytoplasmic domain of unknown function. Colony-stimulating factor 1 (CSF-1) and tumor necrosis factor α are not members of the EGF family but are also synthesized as part of membrane-bound precursors (11, 12). The integral membrane glycoprotein nature of several of these precursors has been biochemically demonstrated (11–15).

Proteolytic processing of these membrane precursors to generate soluble factors is not always an efficient process. Thus, incomplete processing of the EGF precursor leads to its accumulation in mouse kidney (16). Incomplete processing of the TGF-α precursor, pro-TGF-α, occurs in most cell types examined, including tumor-derived cells, retrovirally transformed cells, and cells transfected with a TGF-α gene (17–20), and leads to release of partially processed forms and accumulation of pro-TGF-α on the plasma membrane (18–22). Pro-TGF-α can establish contact with the EGF/TGF-α receptor on the surface of an adjacent cell and activate the receptor-associated tyrosine kinase activity (19, 20) thereby leading to increased calcium uptake (19).

An important question raised by these findings is whether the interaction between the two components of a membrane growth factor receptor system can mediate cell–cell adhesion and, if so, whether this form of cell–cell contact is associated with a mitogenic response. The present study demonstrates the ability of a membrane growth factor precursor and its receptor to function as mediators of cell adhesion and illustrates a mode of mitogenic stimulation that occurs in association with cell–cell contact.

MATERIALS AND METHODS

Cell Lines. GP-TGFα cells and GB1neo^r cells were generated by infection of murine GB1/6 stromal cells with defective retroviruses containing the pZipTGFα vector (23) or the same vector lacking the TGF-α cDNA insert, pZipSV(x) (24). Infected cells were selected for resistance to G418 (1 mg/ml) and used as mass populations. Generation of ZipTGF cells (clone EUT) by introduction of pZipTGFα into NIH 3T3 mouse fibroblasts has been described (23). All these cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 10 μM hydrocortisone sodium succinate. Transfection and selection of interleukin 3 (IL-3)-dependent mouse 32D cells with a retroviral vector containing the human EGF receptor cDNA have been described (25). 32D-EGFR cells respond to EGF at 0.15–5 nM (25). 32D-EGFR cells and parental 32D cells were maintained as suspension cultures in RPMI 1640 with 10% fetal bovine serum and EGF (25) or IL-3 (26).

Metabolic Labeling and Immunoprecipitations. Subconfluent cell monolayers labeled for 20 min with [^{35}S]cysteine (DuPont/New England Nuclear; 300 μCi/ml; 1 μCi = 37 kBq) in cysteine- and serum-free minimum essential medium (MEM) were transferred to complete MEM to provide a nonradioactive "chase." Cell lysis, immunoprecipitation with anti-pro-TGF-α IgG fraction, electrophoresis, and fluorography were done as described (14, 22). For elastase treatment, GP-TGFα cell monolayers labeled with [^{35}S]-cysteine for 20 min and chased for 5 min with complete medium were chilled on ice. Cells were washed with ice-cold MEM and incubated for 1 hr at 4°C in MEM with or without porcine pancreatic elastase (Worthington; 250 μg/ml) and

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Abbreviations: TGF, transforming growth factor; EGF, epidermal growth factor; IL, interleukin; CSF, colony-stimulating factor.

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soybean trypsin inhibitor (Sigma; 250 μg/ml). Incubations were stopped by washing the cells three times with immunoprecipitation buffer. Controls received elastase immediately before the incubation was stopped.

***In Vitro* Engraftment of 32D-EGFR Cells.** For cocultivation studies, confluent monolayers of GP-TGFα or GB1neo^r cells were established in 25-cm^2 flasks or in 9-cm^2 dishes. Suspensions of 32D-EGFR cells or 32D cells were washed three times in serum-free medium and incubated for 1 hr at 37°C. Stromal cell monolayers were then inoculated with 2×10^6 nonadherent cells per flask or 0.7×10^6 cells per dish in RPMI 1640 with 10% fetal bovine serum. At approximately weekly intervals, the culture medium from all flasks was harvested and cell layers were washed and replenished with fresh medium. Nonadherent cells harvested from these cultures were washed and then counted in a hemocytometer. Cell viability was determined by trypan blue exclusion. Foci of adherent hematopoietic cells on the stromal monolayers were counted under phase-contrast microscopy. Two hundred microscopic fields were scored per 25-cm^2 flask (fields per flask ≈1000), and 70 fields were scored per 35-mm dish (fields per dish ≈360).

Mouse EGF (Collaborative Research) or rat fibroblast TGF-α (27) was added to cultures on the indicated days and every 48 hr thereafter until the end of the experiment. Rabbit anti-human EGF receptor antiserum (gift of R. Davis, University of Massachusetts) was added daily to cultures at a final dilution of 1:1000. Normal rabbit serum was used as a control at the same dilution.

Other Assays. To assay TGF-α mRNA levels, poly(A)$^+$ RNA isolated from cells lysed in the presence of proteinase K and SDS (28) was electrophoresed in a 1% agarose gel, blotted onto a nylon membrane, and probed with a human TGF-α cDNA (3) radiolabeled by random priming. Soluble TGF-α in samples of 20-fold concentrated conditioned medium was measured by radioreceptor assay (29). Labeling of nuclei undergoing DNA replication was done as described (30).

RESULTS

Generation of a Stromal Cell Line That Expresses Pro-TGF-α on the Cell Surface. The murine bone marrow stromal cell line GB1/6 supports myelopoiesis of enriched progenitors from long-term bone marrow cultures but does not support adhesion or proliferation of the IL-3-dependent hematopoietic progenitor cell line 32D (26, 31). The GB1/6 derivative GP-TGFα was obtained by infection with vector pZipTGFα, which contains the entire coding region for human pro-TGF-α transcribed under the control of a retroviral long terminal repeat (23). The same vector lacking the pro-TGF-α cDNA insert, pZipSV(x), was introduced into GB1/6 to generate the control cell population, GB1neo^r. Northern blot analysis demonstrated the presence of pro-TGF-α mRNA with the expected size [4.8 kilobases (kb)] in GP-TGFα cells and no detectable expression of the endogenous TGF-α gene in GB1neo^r cells (Fig. 1*A*).

The biosynthetic processing of pro-TGF-α in GP-TGFα cells was evaluated by pulse–chase metabolic labeling experiments. Lysates obtained from these cells were immunoprecipitated with antibodies raised against the C-terminal cytoplasmic sequence of pro-TGF-α (14). Precipitates obtained immediately after the labeling pulse displayed two specifically labeled pro-TGF-α products, of 17 and 21 kDa (Fig. 1*B*). These two products disappeared after a 15-min metabolic chase, concomitantly with the appearance of an 18-kDa labeled product. Based on characterization of similar products from other cell lines that express pro-TGF-α (13, 19, 22), these products were identified as nascent pro-TGF-α (17 kDa), post-Golgi pro-TGF-α (21 kDa), and pro-TGF-α cleaved at the N-terminal domain that precedes the TGF-α sequence in the precursor (18 kDa). Cleavage of pro-TGF-α at the C terminus of the TGF-α polypeptide releases this factor into the medium, leaving the 15-kDa transmembrane/cytoplasmic fragment associated with the cell (13, 19, 22). A labeled 15-kDa immunoreactive product in GP-TGFα cells appeared with very slow kinetics, being detectable as a faint labeled band only after 4 hr of chase (Fig. 1*B*).

Expression of pro-TGF-α in GP-TGFα cells was too low for detection of this molecule on the cell surface by immunofluorescence or by labeling with ^{125}I and immunoprecipitation. To determine whether pro-TGF-α became exposed on the surface of GP-TGFα cells, we tested its susceptibility to elastase, which cleaves at the N terminus of the TGF-α sequence (18). GP-TGFα monolayers radioactively labeled under conditions that preferentially label the 21-kDa pro-TGF-α species were exposed to elastase for 1 hr at 4°C. This treatment quantitatively converted the 21-kDa labeled pro-TGF-α species to an 18-kDa product (Fig. 1*C*). This conversion was not observed in control cultures that received elastase just before the incubation was stopped (data not shown).

These results indicated that pro-TGF-α synthesized in GP-TGFα cells became rapidly exposed on the cell surface and was cleaved at the N terminus. Further processing of the molecule was very slow. Consistent with these results, the concentration of free TGF-α in medium conditioned for 24 hr by GP-TGFα cells was very low, below the detection limit (20

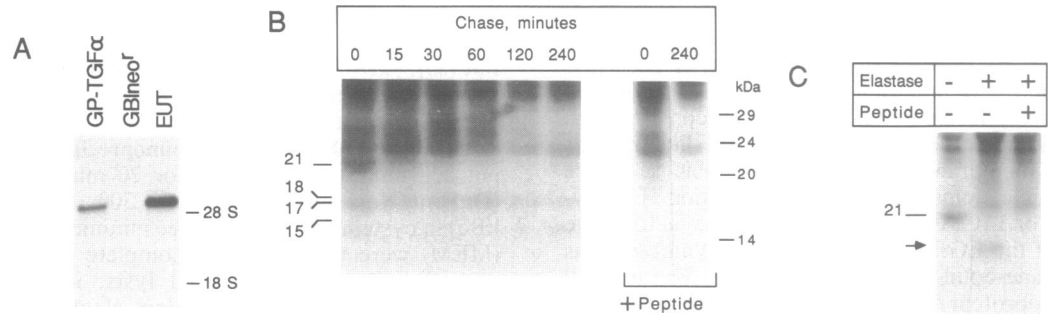

FIG. 1. Expression, biosynthesis, and surface exposure of pro-TGF-α in GP-TGFα cells. (*A*) Northern blot analysis of poly(A)$^+$ RNA (3 μg per lane) from GP-TGFα cells, GB1neo^r cells, and ZipTGF (clone EUT) cells. The probe was a radiolabeled human pro-TGF-α cDNA. Positions of 28S and 18S rRNA are shown. (*B*) Pro-TGF-α biosynthesis. GP-TGFα cells were labeled for 20 min with [^{35}S]cysteine and chased with unlabeled complete medium. Cell lysates were immunoprecipitated with anti-pro-TGF-α antibodies in the absence or presence of the pro-TGF-α C-terminal synthetic peptides against which the antibodies were raised. Immunoprecipitates were resolved by SDS/12–18% PAGE and labeled proteins were detected by fluorography. Sizes of marker proteins are at right, and those of pro-TGF-α (21 kDa) and its products are at left. (*C*) Cleavage of cell surface pro-TGF-α by exogenous elastase. GP-TGFα cell monolayers labeled with [^{35}S]cysteine for 20 min and chased for 5 min with complete medium were chilled on ice and exposed to elastase or medium alone. Cell lysates were immunoprecipitated with anti-pro-TGF-α antibody alone or with excess immunogenic synthetic peptide. Samples were analyzed by electrophoresis and fluorography. The 21-kDa mature pro-TGF-α and the 18-kDa (arrow) product specifically immunoprecipitated after elastase treatment are indicated.

pM) of radioreceptor assay and radioimmunoassay (18). By comparison, EUT cells (23), which express a high level of pro-TGF-α mRNA (Fig. 1*A*), accumulated 15 nM TGF-α in the medium in 24 hr (data not shown).

Adhesion of a Hematopoietic Cell Line Expressing the EGF Receptor to Stromal Cells Expressing Pro-TGF-α. The hematopoietic progenitor cell line 32D is dependent on IL-3 for proliferation and survival and does not respond to other hematopoietic growth factors, including granulocyte/macrophage-CSF and CSF-1 (26). This cell line lacks receptors for EGF but expresses all the components of the intracellular pathway needed to mediate a mitogenic response to this factor, as demonstrated with the EGF receptor-transfected 32D cell clone 32D-EGFR (25). 32D-EGFR cells were cocultivated with confluent monolayers of GP-TGFα stromal cells in the absence of added IL-3 or EGF. Within 4–6 days, the 32D-EGFR cells began to form foci of flattened adherent cells with ≈10 cells per focus (Fig. 2). The morphology of these foci was typical of the "cobblestone islands" generated by primary cultures of bone marrow hematopoietic progenitors and stromal cells (32, 33). The 32D-EGFR cell islands progressively increased in size (>25 cells per island) and number between days 6 and 40 of cocultivation (Fig. 3*A*, bars a). In addition to attachment to the stromal layer, the adherent foci of 32D-EGFR cells were able to continuously release viable hematopoietic cells into the culture medium for at least 40 days in culture (Fig. 3*B*, bars a). We infer that the hematopoietic cells released into the culture medium were derived from the adherent cells, because the medium and nonadherent cells were removed from flasks weekly, and flasks were rinsed and replenished with fresh medium. Cells released into culture medium had the phenotype of normal 32D-EGFR cells (25) as determined by their ability to respond to both EGF and IL-3 and to form colonies in semisolid medium (data not shown). Little or no long-term adherent cell island formation and cell proliferation were detected when 32D-EGFR cells were cocultivated with GB1neo^r cells or when 32D cells were cocultivated with GP-TGFα cells or GB1neo^r cells (Fig. 3, bars b–d).

Adhesion to Stroma Is Mediated by EGF Receptor Binding to Membrane Pro-TGF-α. We tested the ability of EGF and TGF-α to inhibit 32D-EGFR cell binding to GP-TGFα monolayers. Addition of EGF from the first day of cocultivation prevented the formation of 32D-EGFR cell islands (Fig. 4*A*). Similarly, there were no detectable adherent cell islands by day 9 in cocultures supplemented with 12 nM TGF-α (data not shown). As expected from previous results (25), the nonadherent 32D-EGFR cells in the culture medium proliferated in the presence of added EGF (Fig. 4*B*). We also evaluated the ability of exogenous EGF to induce the disappearance of preformed 32D-EGFR cell islands. EGF added to 7-day cocultures of 32D-EGFR and GP-TGFα cells that contained multiple islands markedly decreased the number of islands by 48 hr (Fig. 4*C*). In other experiments (data not shown), cocultures containing 0.1% preimmune serum generated islands of adherent 32D-EGFR cells (40 islands per cm^2) on GP-TGFα monolayers and sustained 32D-EGFR cell proliferation (to 1.4×10^5 cells per dish), whereas no adherent islands were detectable and only 5×10^3 32D-EGFR cells were viable in 7-day cocultures containing 0.1% anti-EGF receptor serum.

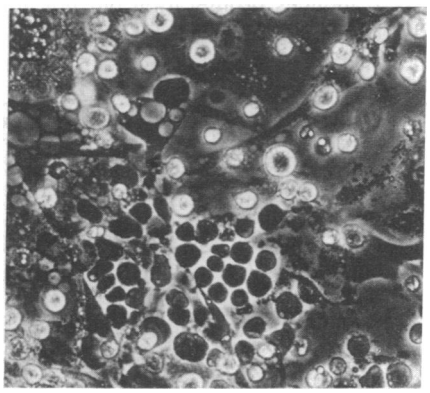

FIG. 2. Adherent 32D-EGFR cell island on a GP-TGFα stromal cell layer. A confluent monolayer of GP-TGFα stromal cells was cocultured with 32D-EGFR hematopoietic progenitor cells. After 10 days, multiple islands of adherent 32D-EGFR cells like the one seen at the bottom were observed. Nonadherent 32D-EGFR cells can be distinguished by their refringence. (×520.)

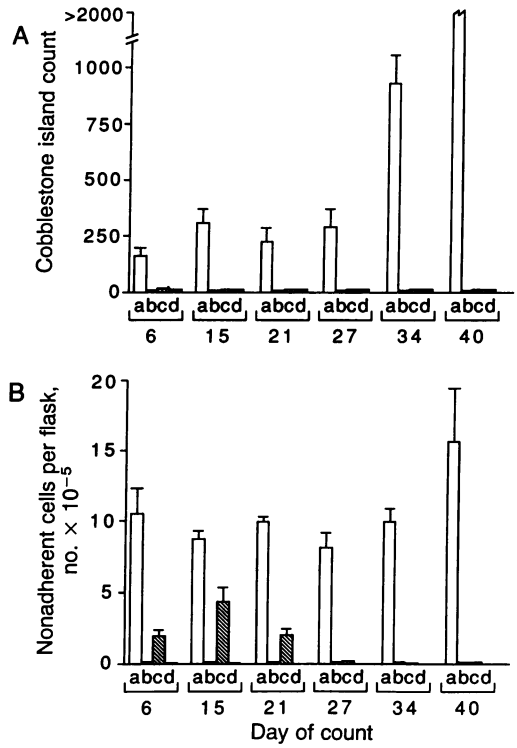

FIG. 3. Adhesion and proliferation of hematopoietic progenitor cells on stromal cell layers. (*A*) 32D-EGFR cells (bars a and b) and parental 32D cells (bars c and d) were cocultivated with GP-TGFα cells (bars a and c) or GB1neo^r cells (bars b and d). At the indicated times after initiation of the cocultivation, the number of adherent cell foci (cobblestone islands) was counted in 200 microscopic fields per flask. (*B*) Media containing nonadherent cells were harvested from the same cocultures on the indicated days, and the adherent monolayers were washed and replenished with fresh medium. Harvested viable nonadherent cells were counted. With the exception of the first harvest (day 6), all nonadherent cells recovered in subsequent harvests were derived from proliferation of adherent progenitors. Data are expressed as mean + SD of three experiments with at least three flasks per experiment.

Proliferation of Hematopoietic Cells Occurs in Contact with Stroma. The sustained increase in 32D-EGFR cell number observed in cocultures with GP-TGFα cells could be due to proliferation of nonadherent cells released from the adherent islands. Alternatively, mitogenic stimulation of 32D-EGFR cells could occur while they were anchored to the monolayers via membrane pro-TGF-α. To distinguish between these two possibilities, 5-bromo-2′-deoxyuridine (BrdUrd) was added to 9-day and 21-day cocultures and allowed to incorporate into replicating DNA. To visualize and quantitate cells that had undergone DNA replication during exposure to BrdUrd, cells were fixed and stained for indirect immunofluorescence with anti-BrdUrd antibody and rhodamine-conjugated secondary antibody. The stromal cells were essentially quies-

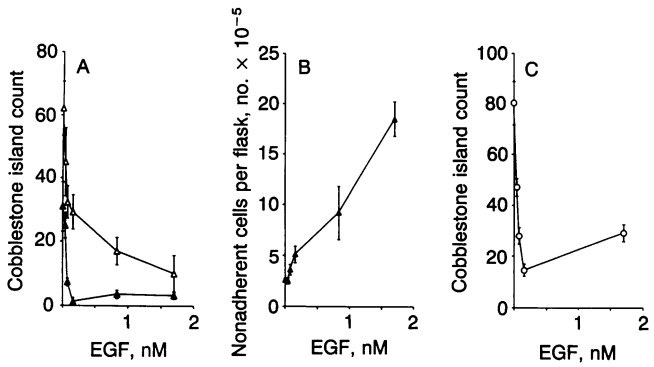

FIG. 4. Inhibition of 32D-EGFR cell adhesion by soluble EGF receptor ligands. EGF was added as indicated to cocultures of 32D-EGFR and GP-TGFα cells from the first day of cocultivation (*A* and *B*) or to 7-day cocultures (*C*). Islands of adherent 32D-EGFR cells (>10 cells per island in 4-day cocultures; >25 cells per island in older cocultures) were counted when cocultures were 4 days (△), 7 days (▲), or 9 days (○) old. Nonadherent 32D-EGFR cells harvested from the cocultures that had been in the presence of EGF for 7 days were counted (*B*). Results are the mean ± SD of two experiments with three flasks per experimental group in each experiment.

cent, with fewer than 0.5% of the nuclei becoming labeled under any of the conditions tested (Fig. 5 and Table 1). A significant proportion of adherent 32D-EGFR cells incorporated BrdUrd into the nucleus (Fig. 5 and Table 1). In 9-day cocultures, this proportion increased progressively with time of exposure to BrdUrd, reaching 38% of the adherent cells after a 24-hr exposure. In 21-day cocultures, as many as 30% of the cells became labeled after only 3 hr of exposure to BrdUrd, but this proportion increased slowly with extended labeling times (Table 1). In contrast to the high labeling index observed in adherent cells, only 10% or fewer of the nonadherent cells recovered from the cocultures became labeled (Table 1). This number included any cells that detached from the monolayers during collection of the medium at the end of the labeling period. Furthermore, removal of the nonadherent cells from the cultures before a short (3 hr) labeling of the cell layers with BrdUrd had essentially no effect on the proportion of adherent 32D-EGFR cells containing labeled nuclei (Table 1). From these results, we conclude that 32D-EGFR cells replicated their DNA while they were bound to membrane pro-TGF-α on the stromal cell monolayer.

DISCUSSION

Cell Adhesion Mediated by a Membrane Growth Factor Binding to Its Receptor. A test system using hematopoietic cells was created to ask whether a membrane growth factor precursor and its receptor can mediate cell–cell adhesion. 32D is an IL-3-dependent cell line that has lost the ability to adhere, proliferate, or survive on GB1/6 mouse stromal cells even though GB1/6 cells support adhesion and proliferation of hematopoietic progenitors from long-term bone marrow cultures (31). By expressing the EGF receptor in 32D cells and pro-TGF-α in GB1/6 cells, we reconstituted the ability of 32D to adhere and proliferate on the stromal cells.

GP-TGFα cells obtained by transfection of GB1/6 with a pro-TGF-α expression vector synthesize pro-TGF-α mRNA and protein but release little if any TGF-α. As previously observed in other cell lines (19, 20, 22), pro-TGF-α reaches the surface of GP-TGFα cells soon after synthesis but remains incompletely processed for several hours thereafter. Several lines of evidence support the conclusion that adhesion of 32D-EGFR cells to GP-TGFα cells is mediated by binding to membrane pro-TGF-α via EGF receptors. (*i*) Formation of adherent progenitor cell islands occurs when

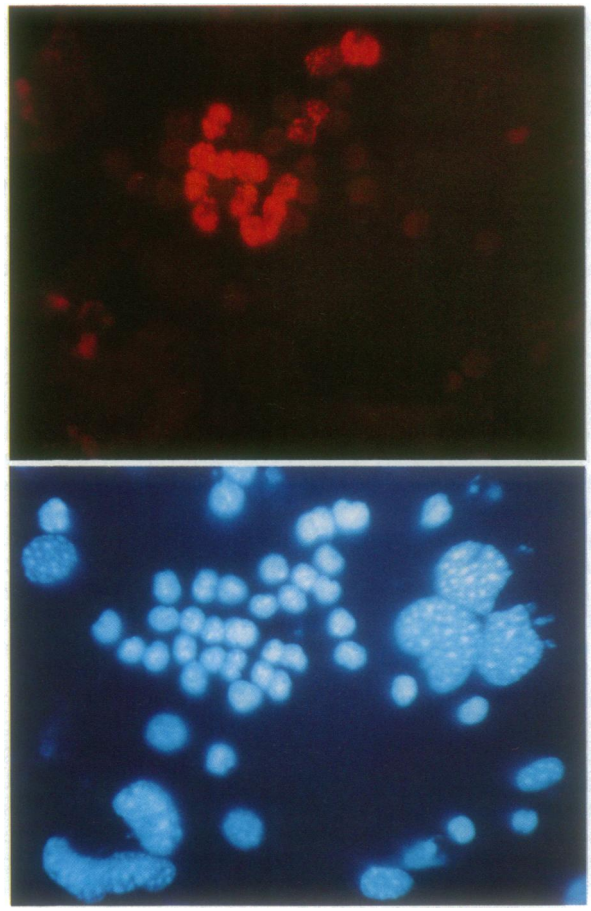

FIG. 5. BrdUrd labeling of replicating nuclei in adherent 32D-EGFR cells. A 9-day coculture of 32D-EGFR progenitor cells and confluent GP-TGFα stromal cells was incubated in the presence of BrdUrd (50 μM) for 9 hr. The adherent cell layer was then washed, fixed, and treated with anti-BrdUrd antibody and rhodamine-conjugated secondary antibody to stain all nuclei that replicated DNA during the labeling period (*Upper*). Finally, all nuclei were stained with bisbenzimide (*Lower*). The photomicrographs correspond to the same field. The larger nuclei correspond to stromal cells, and the small dense nuclei to adherent progenitors.

these two cell lines are cocultured with each other but not when they are cocultured with counterparts that lack the growth factor precursor or the receptor. (*ii*) Adhesion is not secondary for proliferation because IL-3 (data not shown), EGF, or TGF-α induces proliferation but not adhesion. In fact, addition of exogenous EGF or TGF-α inhibits cell island

Table 1. DNA replication in 32D-EGFR cells

Coculture age, days	Exposure to BrdUrd, hr	Labeling index, %*	
		Adherent cells	Nonadherent cells
9	3	8	NT
	9	21	7
	12	25	8
	24	38	6
21	3	30	11
	3†	29	—
	12	37	NT
	24	48	NT

NT, not tested.
*(No. of rhodamine-labeled nuclei/no. of bisbenzimide-labeled nuclei) × 100; 300–400 nuclei were scored per experimental condition.
†The culture medium containing nonadherent cells was removed before the adherent cell layers were labeled with BrdUrd.

formation, presumably by competing for or down-regulating EGF receptors on the progenitor cells. (*iii*) The ability of anti-EGF receptor antibodies to block island formation argues that cell adhesion is mediated by this receptor and not by another pro-TGF-α-binding molecule on the surface of the progenitor cells.

Interaction between pro-TGF-α and EGF receptor appears to be critical not only for establishment of adherent cell islands but also for their long-term maintenance, as suggested by the ability of added EGF to eliminate preformed islands. It remains to be determined why attachment of progenitors occurs in clusters rather than uniformly throughout the stromal layer. We note that island formation is not peculiar to the 32D-EGFR/GP-TGFα cell system but is also observed in primary murine long-term bone marrow cultures and in cocultures of bone marrow stromal cell lines and enriched primary progenitor cells (32, 33). It is possible that additional cell adhesion molecules collaborate with the pro-TGF-α/EGF receptor pair to establish or maintain cell–cell adhesion in our model system.

Proliferation Associated with Cell–Cell Adhesion. 32D-EGFR cell islands attached to GP-TGFα stromal layers proliferate and repopulate the medium with progenitor cells for many weeks in culture. The BrdUrd nuclear labeling experiments show that the progenitor cell population attached to stroma has the highest proportion of mitogenically active cells. This proportion does not vary when labeling is performed after removal of nonadherent cells. Thus, 32D-EGFR cells replicate their DNA while they are bound to stroma via the EGF receptor/pro-TGF-α interaction.

The evidence suggests that pro-TGF-α interaction with EGF receptors generates the primary mitogenic signal in 32D-EGFR cells. Medium conditioned by GB1/6 or GP-TGFα cells is not mitogenic for 32D-EGFR cells. We cannot rule out the possibility that progenitors brought into close contact with stromal cells via the EGF receptor/pro-TGF-α interaction respond to local concentrations of soluble TGF-α or other mitogens released at low levels by the stromal layer. However, uncleaved pro-TGF-α does activate the EGF receptor, leading to activation of calcium uptake (19). Therefore, it is likely that binding of 32D-EGFR cells to membrane pro-TGF-α leads to activation of at least early EGF post-receptor events. Do these events culminate in a mitogenic response without eventual cleavage or dissociation of receptor-bound pro-TGF-α? The low level of pro-TGF-α expression in GP-TGFα cells did not allow us to address this point.

Juxtacrine Stimulation. We propose "juxtacrine" to denote the mode of intercellular stimulation mediated, or at least initiated, by binding of a membrane-anchored growth factor to its receptor on an adjacent cell. The nondiffusible nature of this mode of intercellular stimulation distinguishes it from the endocrine, paracrine, and autocrine stimulation modes (34). The principle of juxtacrine stimulation was suggested by the membrane-anchored nature of precursors for various growth and morphogenesis factors and is substantiated by present and previous (13, 19) studies which show that membrane pro-TGF-α can contact EGF receptors on membranes of adjacent cells.

Juxtacrine stimulation could be a common event in metazoa. It might play an important role in developmental processes based on discrete cell–cell interactions not compatible with the diffusible nature of secretory factors. It might also mediate cell homing to tissue sites rich in receptors, thus targeting the producers of paracrine soluble factors to responsive tissues, and vice versa. In addition to EGF-related factors, other candidate juxtacrine molecules include CSF-1 (G. Stein and C. Rettenmier, personal communication; J.P., unpublished data) as well as tumor necrosis factor α and several receptorlike transmembrane tyrosine kinases with no known soluble ligands (35).

Juxtacrine stimulation could be reciprocal, simultaneously stimulating the two cells involved in it. The possibility that membrane-bound ligands may also have a signaling function is suggested by the EGF-related *lin-12* product, which determines the developmental fate of the cell that expresses it (8). This precedent and the fact that the pro-TGF-α cytoplasmic domain is highly conserved (3, 4) raise the interesting possibility that cells expressing pro-TGF-α might become stimulated via this molecule when they interact with cells that express the EGF receptor.

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